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Apparent Triploidy in the Unisexual Brahminy Blind Snake, *Ramphotyphlops braminus*

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ABSTRACT

Specimens of *Ramphotyphlops braminus* (all females) from Hawaii, the Seychelles, and south Florida, were karyotyped and compared to other typhlopid species. The chromosome number of *R. braminus* is 42, compared to $2n = 32$ for the diploid species *Rhinotyphlops schlegelii* and *Typhlops simoni,* and $2n = 34$ for *Typhlops jamaicensis* and *T. richardi.*

The higher number of chromosomes found in *R. braminus* is best explained by interpreting the karyotype to be triploid, and the chromosomes can easily be grouped into triplets. With the triploid interpretation, few Robertsonian changes are necessary to explain differences between the haploid karyotypes of *R. braminus* and *T. jamaicensis,* with which it is directly compared. Furthermore, the electrophoretic pattern of one dimeric protein, heterozygous in all five individuals from the Seychelles, is best interpreted as having a 1:4:4 pattern, consistent with an interpretation that *R. braminus* is triploid.

Chromosomal heteromorphisms found in all individuals from the three populations are consistent with evidence that *R. braminus* is parthenogenetic, regardless of the interpretation of ploidy level.

INTRODUCTION

Ramphotyphlops braminus is a diminutive blind snake with a worldwide distribution within tropical and subtropical regions. Throughout much of its range, *R. braminus* has likely been accidentally introduced as a result of its close association with humans,

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making *R. braminus* the most widespread terrestrial snake (McDowell, 1974; Nussbaum, 1980). McDowell (1974) proposed that its success in establishing itself so widely might also be explained by a parthenogenetic mode of reproduction.

McDowell (1974) first suggested that *R. braminus* was an all-female species after he failed to find any males in a sample of 114 individuals from across a large portion of the species' range. Nussbaum (1980) corroborated McDowell's findings with a sample of 32 females and no males collected on the four islands from which *R. braminus* is known in the Seychelles: Mahé, Praslin, La Digue, and Fregate. Samples from the Seychelles and elsewhere show low ranges of variation in coloration, number of vertebrae, head scale configuration, and number of longitudinal and transverse scale rows (Nussbaum, 1980). This is additional evidence of a clonal nature for *R. braminus.*

No other evidence supporting parthenogenesis in *R. braminus* is known. Because chromosomal data often provide information on levels of ploidy, mode of reproduction, and origins and relationships of unisexual species (see Darevsky et al., 1985, for a review of the unisexual reptiles), we have investigated the karyotypes of specimens from three populations of *R. braminus*: one in Hawaii, one in the Seychelles, and the other near Miami, Florida. In the absence of other good comparative material, we also describe the karyotypes of the bisexual species *Typhlops jamaicensis* and *T. richardi.*

Acknowledgments

We could not have accomplished this study if friends and colleagues had not kindly provided the living snakes. The sample from Mahé was contributed by Peter Mundell (Division of Arachnids and Myriapods, National Museum of Natural History, Washington, D.C.); the sample from Oahu was furnished by Samuel S. Sweet (University of California, Santa Barbara), who had received them from Sean McKeown (Honolulu Zoo); and our sample from Florida was sent by Julian C. Lee (University of Miami, Florida). S. Blair Hedges (University of Maryland, College Park) provided the *T. jamaicensis* and Richard Thomas (University of Puerto Rico, Rio Piedras) the *T. richardi.* The electrophoretic results reported here were obtained in the laboratory of Richard Highton (University of Maryland, College Park), who provided facilities and financial support (NSF grant DEB 83-07115 and NSF grant BSR-85-07847 to Richard Highton). We greatly appreciate the efforts extended by all of these people and by Carol R. Townsend (American Museum of Natural History), who assisted with some of the chromosomal preparations. We also thank Herbert C. Dessauer and Laurence M. Hardy for their helpful comments on the manuscript.

MATERIALS AND METHODS

The specimens of *R. braminus* we examined are from Victoria, Mahé, the Seychelles (USNM 258097 and 258098); Kapiolani Park, Honolulu Zoo, Honolulu, Oahu, Hawaii (AMNH 129740, 129742, 129743, and 129750); and 1 mi west of the University of Miami campus, Coral Gables, Florida (USNM 259179 and 260774). The *T. jamaicensis* (USNM 259180) is a female from 2.1 km east of St. Anns Bay, St. Anns Parish, Jamaica, and the *T. richardi* (USNM 258101) is a male from 6 km SSE of Dorado, Puerto Rico.

Chromosomal preparations of the *R. braminus* from Mahé and Florida were obtained from the whole animal, except for the head, tail (including cloacal region), and interconnecting skin, which were preserved as a voucher specimen for each animal. Only liver was used from the *T. jamaicensis,* and liver, spleen, kidney, bone marrow, and testes were used from the *T. richardi.* Each specimen was pretreated with colchicine. The body parts were minced in 0.8 percent sodium citrate at room temperature. After 15 minutes, the material was strained through gauze and the cell-bearing suspension was centrifuged. Thereafter, the method followed that of Patton (1967). In addition, two of the *R. braminus* (USNM 258098 and 259179), the *T. jamaicensis,* and the *T. richardi* were injected with phytohemagglutinin two to four days before being sacrificed. Chromosomes of the *R. braminus* from Oahu were prepared similarly, using phytohemagglutinin, colchicine, minced

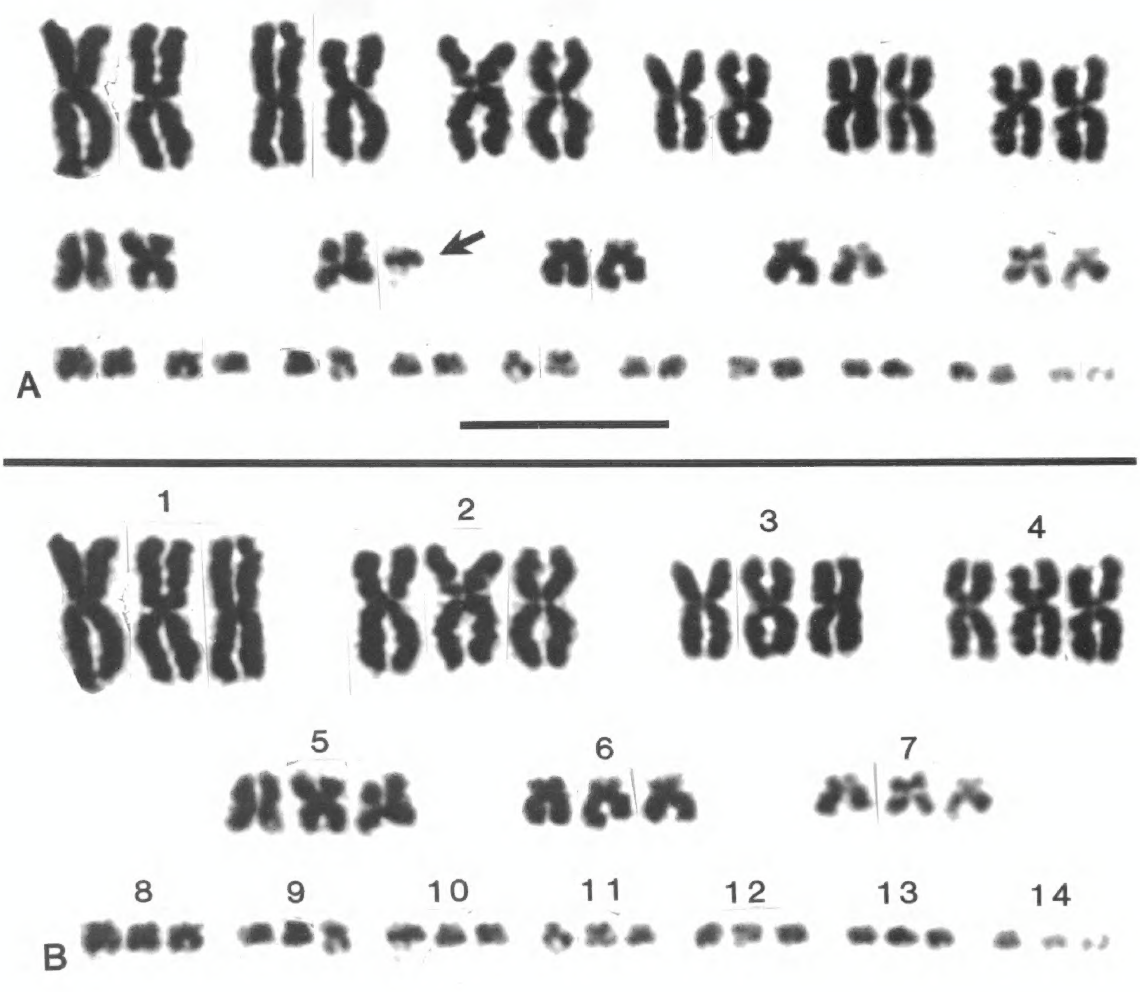

Fig. 1. The 42 chromosomes of *Ramphotyphlops braminus* (AMNH 129743; female), from one somatic cell at metaphase. **A.** Diploid interpretation. The arrow shows a microchromosome with a secondary constriction. The line represents 10 μm. **B.** Triploid interpretation of the same chromosomes.

vertebrae and ribs, and a 30-minute soak in 1.0 percent sodium citrate.

RESULTS AND DISCUSSION

Our analysis is based on the examination of nine dividing cells from the two individuals from Mahé, 15 cells from the 4 specimens from Oahu, and 26 cells from the 2 snakes from Florida. The specimens from Mahé and Oahu are from larger series, a total of 5 from Mahé (USNM 258096 to 258100) and 12 from Oahu (AMNH 129740 to 129751). Although one specimen from Oahu could not be sexed due to the poor state of preservation, the remaining 16 in both samples were determined to be female by the presence of a right oviduct and/or ovarian follicles. This provides evidence that *R. braminus* from Oahu is also all-female and supports the findings of Nussbaum (1980) and McDowell (1974). The two animals from Coral Gables are also females.

Individuals from all three populations have chromosomal counts of 42 (21 macrochromosomes and 21 microchromosomes), the chromosomal complements being identical in both size and shape, as well as number (fig. 1). The macrochromosomes can be divided

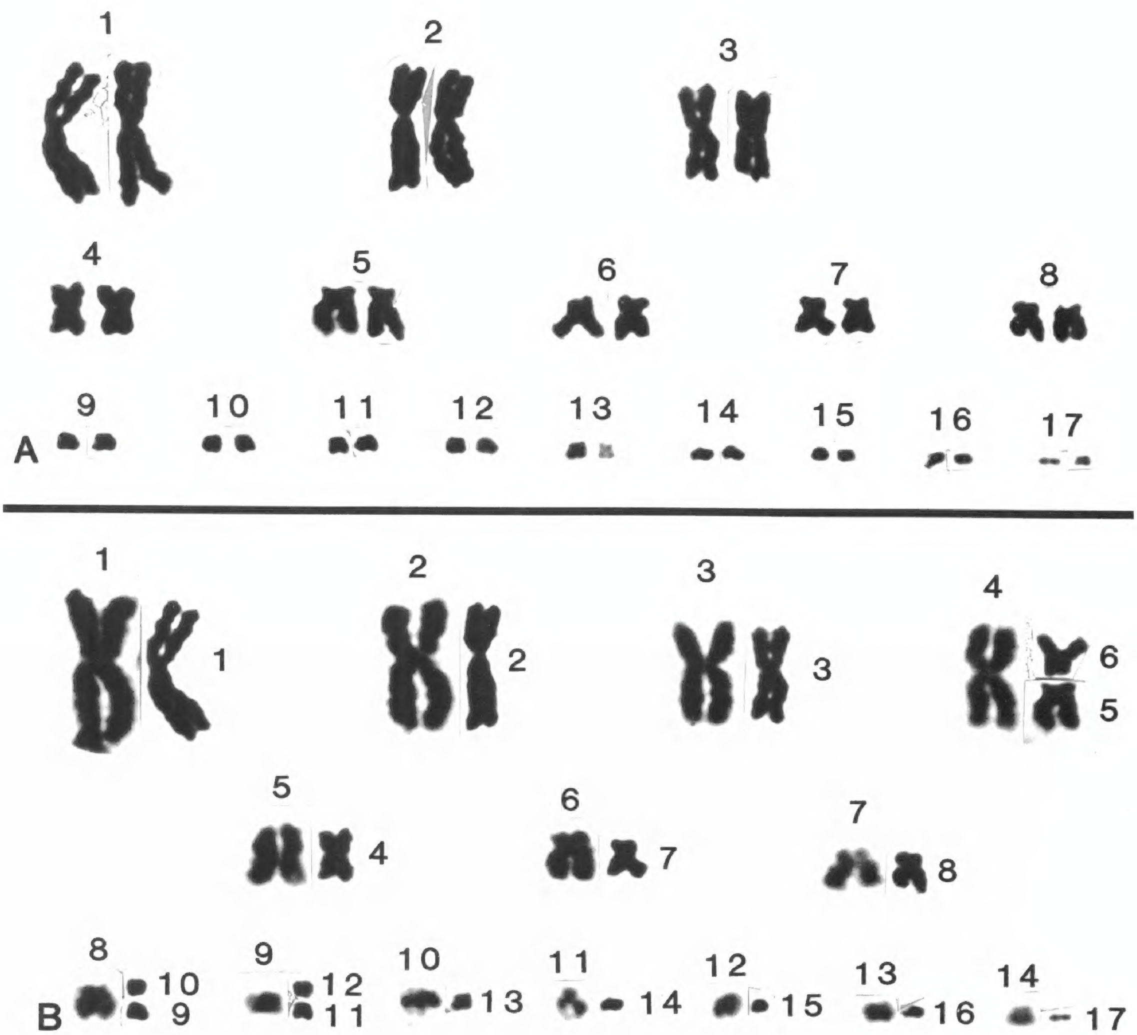

Fig. 2. Chromosomes of two typhlopid snakes, from somatic cells at metaphase. **A.** Diploid karyotype ($2n = 34$) of the bisexual *Typhlops jamaicensis* (USNM 259180; female). *Typhlops richardi* has identical chromosomes. **B.** One haploid set of chromosomes of *T. jamaicensis* (right chromosome(s) of each "pair"; from A) "paired" with one chromosome of each triplet of *R. braminus* from fig. 1B (left chromosome of each "pair").

into four general groups, best visualized in figure 1B. The first three groups are composed of six metacentric, six metacentric, and three metacentric chromosomes, respectively, in decreasing order of size. The fourth and smallest group consists of one submetacentric chromosome and five telo- to subtelocentric chromosomes. In many cells, several microchromosomes appear to be biarmed. Telo- and subtelocentric microchromosomes are also apparent, one of which consistently has a secondary constriction (fig. 1A). These chromosomes can be interpreted as representing either a diploid or triploid condition (see below; also fig. 1A, B).

In contrast, both *T. jamaicensis* and *T. richardi* have diploid numbers of 34 (fig. 2A), with 16 macrochromosomes, two of which

are submetacentric (pair 2), six metacentric (pairs 1, 3, and 4), and eight subtelocentric (pairs 5 through 8), plus 18 microchromosomes, several of which are biarmed. These karyotypes seem similar to those of the only two other typhlopids for which chromosomes have been described (but not illustrated). A female *Rhinotyphlops schlegelii* was reported by Fischman et al. (1972) to have 32 chromosomes (14 metacentric and 2 submetacentric macrochromosomes, plus 16 microchromosomes). Werner (1959) gave a count of 32 (10 metacentric and 6 telocentric macrochromosomes, plus 16 microchromosomes) for a male *Typhlops simoni.* The karyotypes of these four bisexual species from widely separated populations are similar, with variation occurring in number of microchromosomes and in the morphology (but not number) of the macrochromosomes.

The karyotypes of the bisexual species (e.g., fig. 2A) provide perspective for interpreting the ploidy level of *R. braminus.* Without evidence to the contrary, a diploid interpretation would be appealing for simplicity (all other snakes analyzed are diploid). There are several problems, however, with a diploid interpretation, as shown in figure 1A. *Ramphotyphlops braminus* has a much higher number of macrochromosomes than any other typhlopid (21 compared to 16 in the others), which cannot be easily accounted for by centric fusion or dissociation using the diploid interpretation. Also problematic with the diploid interpretation is a heteromorphic pair, consisting of one macrochromosome and one microchromosome by our interpretation (the pair with the arrow in fig. 1A). A heteromorphic pair has not been observed in any of the other four typhlopids karyotyped. There is also a small but consistent size difference between the two chromosomes in the second largest pair (fig. 1A).

If *R. braminus* is interpreted as being triploid (fig. 1B), its diploid ancestor(s) could be inferred to have been $2n = 28$ (14 macrochromosomes and 14 microchromosomes). The differences in numbers and morphology of macrochromosomes and microchromosomes, when compared to the other typhlopids of known karyotype, are readily accounted for by only three Robertsonian changes (whole-arm translocations), one among the macrochromosomes and two among the microchromosomes. This can be seen in a photographic composite of a haploid complement of *T. jamaicensis* chromosomes and a haploid complement (assuming triploidy) from *R. braminus* (fig. 2B). The correspondence between the typhlopid chromosomes here is much closer than is seen if a diploid interpretation is used for *R. braminus.*

With a triploid interpretation for *R. braminus,* the only discrepancy in chromosome size or shape within a group is in set 7 (fig. 1B), which has a small submetacentric and two small subtelocentric macrochromosomes. Similarly, this small submetacentric chromosome must be paired with a subtelocentric chromosome with a diploid interpretation (the smallest pair of macrochromosomes in fig. 1A). This heteromorphism may reflect a chromosomal difference between two ancestral species if *R. braminus* is hybrid in origin or has had a hybrid event subsequent to becoming parthenogenetic. Alternatively, the heteromorphism could have resulted from a single nonlethal chromosomal aberration after establishment of parthenogenesis, as has been observed in clones of *Cnemidophorus exsanguis* (see Cole, 1979).

Darevsky et al. (1985) noted that evidence of prior hybridization is present in all well-studied taxa of parthenogenetic vertebrates. The presence of only one secondary constriction in the karyotype of *R. braminus* (fig. 1A, arrow; 1B, left chromosome in set 10) may implicate hybridization in the ancestry of these triploids. Diploid bisexual species of reptiles analyzed so far usually have only one pair of active nucleolar organizer regions (NORs) on homologous chromosomes, and these are often (not always) correlated with the consistent secondary constrictions observed in standard preparations (Bickham and Rogers, 1985; Ward and Cole, 1986). However, in parthenogenetic lizards of hybrid origin, frequently one or two (in triploids) of the NORs are inactivated through nucleolar dominance (Ward and Cole, 1986).

Ascertaining the karyotypes of other species of *Ramphotyphlops* would help to test our hypothesis that *R. braminus* is triploid and possibly hybrid, and may help to locate the ancestral populations. In addition, comparative analyses of tissue proteins may prove

to be more sensitive than karyotypic analyses in addressing these questions.

A preliminary electrophoretic survey for protein variation at presumptive gene loci in the five specimens of *R. braminus* from Mahé also provides evidence that *R. braminus* is triploid. Of eight loci clearly resolved, one of two malate dehydrogenase isozymes (MDH, obtained with tris-citrate buffer at a pH of 6.7) appeared heterozygous for the same two alleles in all five specimens. Other species of *Typhlops* have two MDH isozymes, one occurring cathodal to, or at, the origin, the other anodal to the origin (AHW, unpub. data; S. Blair Hedges and Richard Thomas, personal commun.). In the five specimens of *R. braminus,* a cathodally occurring band was interpreted to represent the cathodal locus, homozygous in all five individuals. An anodal protein had a three-banded pattern, interpreted as showing all five specimens to be heterozygous at this locus. It did not show a 1:2:1 ratio of banding intensities, as would be predicted for a dimeric protein if one copy of each of two different alleles was present. Instead, it had a pattern which could be interpreted as a 1:4:4 ratio, the most anodal band being the faintest, suggesting that two copies of one allele were present with one copy of another (genotype *a b b*). Such results should be viewed with caution, however, as 1:2:1 banding intensities do not always occur as predicted in diploid organisms, and secondary isozymes can often produce multibanded patterns in homozygous individuals. Additional research should also be conducted to determine whether this heterozygosity is fixed, as suggested by our limited sample.

Our specimens are from widely separated areas (the Seychelles, Hawaii, and south Florida), which might indicate that the karyotype of *R. braminus* is similar over a large portion of its range. This conclusion may not be correct, however, considering the extent to which *R. braminus* has been transported worldwide and that each population we studied was introduced. Honegger (1966) reported that *R. braminus* was introduced into the Seychelles between 1936 and 1939, but he did not give the origin of the introduced animals. The population from Oahu apparently originated from animals accidentally brought from the Philippines prior to 1930 (Tinker, 1938; Oliver and Shaw, 1953). *Ramphotyphlops braminus* has only recently been found to occur in Florida (Wilson and Porras, 1983).

In conclusion, the karyotypes are best interpreted as showing that the *R. braminus* we sampled are triploid, as are many parthenogenetic species. Even if interpreted as diploid, the apparently fixed karyotypic heterozygosity provides evidence of clonal inheritance in *R. braminus.* As Nussbaum (1980) suggested, several species, both bisexual and unisexual, could conceivably compose a species complex that is referred to presently as *R. braminus.* It will be interesting to determine whether the karyotypic uniformity we have observed is found in other populations, and whether all populations identified as *R. braminus* are similarly triploid.

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